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HUMAN CELL SURFACE RECEPTOR PROTEINS

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TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of human cell surface receptor proteins and to the use of these sequences in the diagnosis, treatment, and prevention of cell proliferative disorders, immune system disorders, infections, and neuronal disorders.

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BACKGROUND OF THE INVENTION

The term receptor describes proteins that specifically recognize other molecules. The category is broad and includes proteins with a variety of functions. The bulk of receptors are cell surface proteins which bind extracellular ligands and produce cellular responses in the areas of growth, differentiation, endocytosis, exocytosis and immune response. Central to the function of cell surface receptors is the capacity to adhere or bind to other proteins or ligands through special functional domains.

Cell surface receptors are typically integral plasma membrane proteins. These receptors recognize hormones such as catecholamines; peptide hormones; growth and differentiation factors; small peptide factors such as thyrotropin releasing hormone; galanin, somatostatin, and tachykinins; and circulatory system-borne signaling molecules. Cell surface receptors on immune system cells recognize antigens, antibodies, and major histocompatibility complex (MHC)-bound peptides. Other cell surface receptors bind ligands to be internalized by the cell. This receptor-mediated endocytosis functions in the uptake of low density lipoproteins (LDL), transferrin, glucose- or mannose-terminal glycoproteins, galactose-terminal glycoproteins, immunoglobulins, phosphovitellogenins, fibrin, proteinase-inhibitor complexes, plasminogen activators, and thrombospondin (Lodish, H. et al. (1995) Molecular Cell Biology, Scientific American Books, New York NY, p. 723; and Mikhailenko, I. et al. (1997) J. Biol. Chem. 272:6784-6791).

Many growth factor receptors, including receptors for epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, as well as the growth modulator α -thrombin, contain intrinsic protein kinase activities. When growth factor binds to the receptor, it triggers the autophosphorylation of a serine, threonine, or tyrosine residue on the receptor. These phosphorylated sites are recognition sites for the binding of other cytoplasmic signaling proteins. These proteins participate in signaling pathways that eventually link the initial receptor activation at the cell surface to the activation of a specific intracellular target molecule. In the case of tyrosine residue autophosphorylation, these signaling proteins contain a common domain referred to as a src homology 2 (SH2) domain. SH2 domains are found in a variety of signaling molecules and oncogenic proteins

such as phospholipase C- γ , Ras GTPase activating protein, and pp60^{c-src} (Lowenstein, E.J. et al. (1992) Cell 70:431-442).

G-protein coupled receptors (GPCRs)

G-protein coupled receptors (GPCRs) are integral membrane proteins characterized by the presence of seven hydrophobic transmembrane domains which span the plasma membrane and form a bundle of antiparallel alpha (α) helices. These proteins range in size from under 400 to over 1000 amino acids (Strosberg, A.D. (1991) Eur. J. Biochem. 196:1-10; Coughlin, S.R. (1994) Curr. Opin. Cell Biol. 6:191-197). The amino-terminus of the GPCR is extracellular, of variable length and often glycosylated; the carboxy-terminus is cytoplasmic and generally phosphorylated. Extracellular loops of the GPCR alternate with intracellular loops and link the transmembrane domains. The most conserved domains of GPCRs are the transmembrane domains and the first two cytoplasmic loops. The transmembrane domains account for structural and functional features of the receptor. In most cases, the bundle of alpha helices forms a binding pocket. In addition, the extracellular N-terminal segment or one or more of the three extracellular loops may also participate in ligand binding. Ligand binding activates the receptor by inducing a conformational change in intracellular portions of the receptor. The activated receptor, in turn, interacts with an intracellular heterotrimeric guanine nucleotide binding (G) protein complex which mediates further intracellular signaling activities, generally the production of second messengers such as cyclic AMP (cAMP), phospholipase C, inositol triphosphate or ion channel proteins (Baldwin, J.M. (1994) Curr. Opin. Cell Biol. 6:180-190).

One group of GPCRs are the rhodopsin-like GPCRs that transmit extracellular signals of diverse natures including hormones, neurotransmitters and light. Rhodopsin is a photosensitive GPCR in the vertebrate eye. Rhodopsin, which defines a conserved subfamily of GPCRs found in animal retinas, is about 350 amino acids in length. In vertebrates, rhodopsin molecules are embedded in membranous stacks found in photoreceptor (rod) cells. Each rhodopsin molecule responds to a photon of light by triggering a decrease in cGMP levels which leads to the closure of plasma membrane sodium channels. In this manner, a visual signal is converted to a neural impulse. Other rhodopsin-like GPCRs are directly involved in responding to neurotransmitters. These GPCRs include the receptors for adrenaline (adrenergic receptors), acetylcholine (muscarinic receptors), adenosine, galanin, and glutamate (N-methyl-D-aspartate/NMDA receptors). (Reviewed in Watson, S. and Arkinstall, S. (1994) The G-Protein Linked Receptor Facts Book, Academic Press, San Diego, CA, pp. 7-9, 19-22, 32-35, 130-131, 214-216, 221-222; Habert-Ortoli, E. et al. (1994) Proc. Natl. Acad. Sci. USA 91:9780-9783.)

The somatostatin receptor type 4 is another example of a rhodopsin-like GPCR. It is one of several high affinity receptors for somatostatin, a tetrapeptide that inhibits the secretion of growth

hormone from the anterior pituitary. Expression of particular somatostatin receptors has been linked to the efficacy of drug therapy in specific endocrine tumors (Kubota, A. et al. (1994) J. Clin. Invest. 93:1321-1325).

Another rhodopsin-like GPCR is the prostanoid EP1 receptor that recognizes prostanoids such as prostaglandin to mediate a variety of physiological functions including cardiovascular and immune responses. EP1 receptors have a role in the contraction and relaxation of smooth muscle and can activate the phosphoinositide pathway (Watson, supra, pp. 239-251). The prostanoid DP receptor is another rhodopsin-like GPCR that is specific for prostaglandin D2 (PGD2). Expression of the DP receptor has been localized to the mammalian brain and eye tissues and upon activation facilitates elevation of intracellular cAMP and Ca²⁺ mobilization but does not generate inositol 1,4,5-triphosphate (Boie, Y. et al. (1995) J. Biol. Chem. 270:18910-18916; Gerashchenko, D. et al. (1998) J. Neurochem. 71:937-945).

Still another rhodopsin-like GPCR is the endothelin receptor that plays a role in cardiovascular system regulation through endothelins. Endothelins are potent vasoconstrictors that can stimulate cardiac and smooth muscle contraction as well as stimulate secretion in tissues such as kidney, liver and adrenals. Endothelin receptors may have a role in the brain, where they are also found, and there is evidence that endothelins may be associated with pathophysiological conditions such as stress (Watson, supra, pp. 111-116).

The secretin receptor is an example of a unique GPCR that responds to secretin, a peptide hormone that stimulates the secretion of enzymes and ions in the pancreas and small intestine (Watson, supra, pp. 278-283). Secretin receptors are about 450 amino acids in length and are found in the plasma membrane of gastrointestinal cells. Binding of secretin to its receptor stimulates the production of cAMP. An unusual member of the secretin receptor family has been identified from a neuroectodermal cDNA library (Baud, V. et al. (1995) Genomics 26:334-344). This receptor, EMR1 (EGF-like, mucin-like hormone receptor), is 886 amino acids in length and contains six epidermal growth factor (EGF)-like modules at the N-terminus followed by a serine/threonine rich domain. The latter feature is characteristic of mucin-like integral membrane adhesive proteins.

Other GPCRs have been identified which play a role in the immune response. For example, a new subfamily of GPCRs has been identified from a human monocyte (HM) cDNA library (Nomura, H. et al. (1993) Int. Immunol. 5:1239-1249). Most of these GPCRs likely bind to cytokines and other leukocytic signaling molecules. One of these GPCRs, HM74, is particularly unusual in that its N-terminus does not contain N-glycosylation sites.

The thrombin receptors (TRs) have GPCR activity and are activated by the ligand α -thrombin. Through TR-mediated signal transduction pathways, α -thrombin induces production of IL-8 and IL-6

in cultured monocytes and endothelial cells (Johnson, K. et al. (1998) J. Immunol. 160:5130-5135). Conversely, α -thrombin inhibits the action of IL-6, leukemia inhibitory factor, and ciliary neurotrophic factor in chinese hamster lung fibroblasts (Bhat, G.J. et al. (1998) Arch. Biochem. Biophys. 350:307-314). In addition, when α -thrombin binds to the TR it proteolytically cleaves 40 amino acids from the N-terminus of the receptor. The cleaved peptide is termed the thrombin receptor agonist peptide and acts as a tethered ligand for the TR to increase the potency of the thrombin-derived signal (Hou, L. Et al. (1998) J. Periodontal Res. 33:205-211; Johnson, et al. supra).

GPCR mutations, which may cause loss of function or constitutive activation, have been associated with numerous human diseases (Coughlin, supra). For instance, retinitis pigmentosa may arise from mutations in the rhodopsin gene. Parma, J. et al. (1993, Nature 365:649-651) report that somatic activating mutations in the thyrotropin receptor cause hyperfunctioning thyroid adenomas and suggest that certain GPCRs susceptible to constitutive activation may behave as protooncogenes.

Cytokine Receptors

Cytokines comprise a family of signaling molecules that modulate the immune system and the inflammatory response. Cytokines are usually secreted by leukocytes, or white blood cells, in response to injury or infection. However, other tissues are capable of secreting cytokines in response to disease or other physiologic perturbations. Cytokines function as growth and differentiation factors that act primarily on cells of the immune system such as lymphocytes, monocytes, and granulocytes. Like other signaling molecules, cytokines bind to specific plasma membrane receptors and trigger intracellular signal transduction pathways which regulate gene expression, cell proliferation, and cell differentiation.

Erythropoietin (EPO) is an unusual cytokine that is produced not by leukocytes, but instead by the kidney or liver. EPO stimulates erythroid precursors to differentiate into red blood cells. EPO also stimulates the production of platelets. The EPO receptor is a single-pass transmembrane protein of about 500 amino acids, the intracellular domain of which associates with JAK2 kinase. Activated EPO receptor stimulates the phosphorylation activity of JAK2 which triggers gene transcription and mitogenesis. (Reviewed in Callard, R. and Gearing, A. (1994) The Cytokine Facts Book, Academic Press, San Diego, CA, pp.114-118.)

Immunoglobulin Domain Receptors

Immune system and related cell surface receptor proteins have hallmark functional domains (for example the Immunoglobulin (Ig) domain) with similar functionality in a wide array of receptor types. The human immune system is responsible for combating infectious agents contracted from the environment. One critical component of the immune system are highly specialized molecules called immunoglobulins (Ig) or antibodies that can recognize and bind to foreign antigens, thereby

facilitating their elimination. Characteristic features of immunoglobulins include their structural motifs that include regions for membrane attachment, antigen recognition (variable (V) regions), and polymerization. Polymerized immunoglobulins such as glandularly secreted IgA and IgM must undergo transcellular transport, a process mediated by the poly-immunoglobulin (poly-Ig) receptor.

5 The poly-Ig receptor is itself a member of the immunoglobulin superfamily having homology to the variable (V) region of immunoglobulins (Hood, L. et al. (1985) Cell 40:225-229). Like all immunoglobulin superfamily members, poly-Ig receptor is involved in adhesion or binding to other proteins through the conserved immunoglobulin-like domain. This Ig domain is comprised of antiparallel β sheets joined by a disulfide bond in an arrangement called the Ig fold. Members of the

10 Ig superfamily include T-cell receptors, MHC proteins, CD4, CD8, and CD 28 cell surface proteins, and antibodies.

Immunoglobulins, or antibodies, are the central components of the humoral immune response. IgG, the most common class of immunoglobulin in the circulation, can be described in terms of two main functional domains. Antigen recognition is mediated by the Fab (antigen binding fragment)

15 region of the IgG, while effector functions are mediated by the Fc (crystallizable fragment) region. Binding of IgG to an antigen, such as a bacterium, triggers the destruction of the antigen by phagocytic white blood cells, such as macrophages and neutrophils. These cells express cell surface receptors that specifically bind to the IgG Fc region and allow the phagocytic cells to engulf, ingest, and degrade the IgG-bound antigen. The IgG Fc receptors expressed by phagocytic cells are single-

20 pass transmembrane glycoproteins of about 400 amino acids (Sears, D. W. et al. (1990) J. Immunol. 144:371-378). The extracellular portion of the IgG Fc receptor typically contains two or three Ig domains.

T cells play a dual role in the immune system as effectors and regulators, coupling antigen recognition with the transmission of signals that induce cell death in infected cells and stimulate other

25 immune cells. Although T cells collectively recognize a wide range of different antigens, a clonal line of T cells can only recognize a single antigen. Moreover, the antigen must be presented to the T cell receptor (TCR) as a peptide complexed with a major histocompatibility molecule (MHC) on the surface of an antigen-presenting cell. The TCR on most T cells consists of two polypeptide subunits, α and β , which are immunoglobulin-like integral membrane glycoproteins of similar molecular

30 weight. The TCR α and TCR β subunits have an extracellular domain containing both variable and constant regions, a transmembrane domain that traverses the membrane once, and a short intracellular domain (Saito, H. et al. (1984) Nature 309:757-762). The genes for the TCR subunits are constructed through somatic rearrangement of different gene segments. Interaction of antigen in the proper MHC context with the TCR initiates signaling cascades that induce the proliferation, maturation, and

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SUMMARY OF THE INVENTION

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selected from the group consisting of SEQ ID NO:1-13 and fragments thereof.

The invention also provides a method for detecting a polynucleotide in a sample containing nucleic acids, the method comprising the steps of: (a) hybridizing the complement of the polynucleotide sequence to at least one of the polynucleotides of the sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide in the sample. In one aspect, the method further comprises amplifying the polynucleotide prior to hybridization.

The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:14-26 and fragments thereof. The invention further provides an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide sequence selected from the group consisting of SEQ ID NO:14-26 and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:14-26 and fragments thereof.

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-13. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing a polynucleotide of the invention under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-13 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide selected from the group consisting of SEQ ID NO:1-13 and fragments thereof. The invention also provides a purified agonist and a purified antagonist to the polypeptide.

The invention also provides a method for treating or preventing a disorder associated with decreased expression or activity of HCSR, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-13 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention also provides a method for treating or preventing a disorder associated with increased expression or activity of HCSR, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-13 and fragments thereof.

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BRIEF DESCRIPTION OF THE TABLES

Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding HCSR.

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Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of HCSR.

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Table 3 shows selected fragments of each nucleic acid sequence: the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding HCSR were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze HCSR, along with applicable descriptions, references, and threshold parameters.

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DESCRIPTION OF THE INVENTION

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Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

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It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be

used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"HCSR" refers to the amino acid sequences of substantially purified HCSR obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

10 The term "agonist" refers to a molecule which intensifies or mimics the biological activity of HCSR. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HCSR either by directly interacting with HCSR or by acting on components of the biological pathway in which HCSR participates.

An "allelic variant" is an alternative form of the gene encoding HCSR. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding HCSR include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as HCSR or a polypeptide with at least one functional characteristic of HCSR. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding HCSR, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HCSR. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HCSR. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of HCSR is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine.

Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

“Amplification” relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term “antagonist” refers to a molecule which inhibits or attenuates the biological activity of HCSR. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HCSR either by directly interacting with HCSR or by acting on components of the biological pathway in which HCSR participates.

The term “antibody” refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind HCSRPs polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term “antigenic determinant” refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term “antisense” refers to any composition containing a nucleic acid sequence which is complementary to the “sense” strand of a specific nucleic acid sequence. Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the

complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand.

5 The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HCSRPs, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

10 The terms "complementary" and "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which
15 depend upon binding between nucleic acid strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution.
20 Compositions comprising polynucleotide sequences encoding HCSRPs or fragments of HCSRPs may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

25 "Consensus sequence" refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using the XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of one or more Incyte Clones and, in some cases, one or more public domain ESTs, using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison
30 WI). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded

as conservative amino acid substitutions.

| | Original Residue | Conservative Substitution |
|----|------------------|---------------------------|
| | Ala | Gly, Ser |
| | Arg | His, Lys |
| 5 | Asn | Asp, Gln, His |
| | Asp | Asn, Glu |
| | Cys | Ala, Ser |
| | Gln | Asn, Glu, His |
| | Glu | Asp, Gln, His |
| 10 | Gly | Ala |
| | His | Asn, Arg, Gln, Glu |
| | Ile | Leu, Val |
| | Leu | Ile, Val |
| | Lys | Arg, Gln, Glu |
| 15 | Met | Leu, Ile |
| | Phe | His, Met, Leu, Trp, Tyr |
| | Ser | Cys, Thr |
| | Thr | Ser, Val |
| | Trp | Phe, Tyr |
| 20 | Tyr | His, Phe, Trp |
| | Val | Ile, Leu, Thr |

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "fragment" is a unique portion of HCSR or the polynucleotide encoding HCSR which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues

in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

5 A fragment of SEQ ID NO:14-26 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:14-26, for example, as distinct from any other sequence in the same genome. A fragment of SEQ ID NO:14-26 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:14-26 from related
10 polynucleotide sequences. The precise length of a fragment of SEQ ID NO:14-26 and the region of SEQ ID NO:14-26 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-13 is encoded by a fragment of SEQ ID NO:14-26. A fragment of SEQ ID NO:1-13 comprises a region of unique amino acid sequence that specifically identifies
15 SEQ ID NO:1-13. For example, a fragment of SEQ ID NO:1-13 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-13. The precise length of a fragment of SEQ ID NO:1-13 and the region of SEQ ID NO:1-13 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

20 The term "similarity" refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization
25 assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to
30 one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and
 5 therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in
 10 Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequence pairs.

15 Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at <http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis
 20 programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST
 25 programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Reward for match: 1

30 *Penalty for mismatch: -2*

Open Gap: 5 and Extension Gap: 2 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 11

Filter: on

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the hydrophobicity and acidity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Open Gap: 11 and Extension Gap: 1 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 3

Filter: on

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

“Human artificial chromosomes” (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term “humanized antibody” refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

“Hybridization” refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of identity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the “washing” step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Generally, such wash temperatures are selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization are well known and can be found in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention



15

The words “insertion” and “addition” refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

20

The term “microarray” refers to an arrangement of distinct polynucleotides on a substrate.

25

The term “modulate” refers to a change in the activity of HCSR. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of HCSR.

30

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably

linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

5 "Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

10 "Probe" refers to nucleic acid sequences encoding HCSRPs, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target
15 DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100,
20 or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold
25 Spring Harbor Press, Plainview NY; Ausubel et al., 1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis et al., 1990, PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

30 Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the

PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, *supra*. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding HCSR, or fragments thereof, or HCSR itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a

protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide
5 containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which
10 they are naturally associated.

A "substitution" refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers,
15 microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

"Transformation" describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic
20 acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently
25 transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-
30 1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding

polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene
5 between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having
10 at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

15

THE INVENTION

The invention is based on the discovery of new human cell surface receptor proteins (HCSRPs), the polynucleotides encoding HCSRPs, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative disorders, immune system disorders, infections, and
20 neuronal disorders.

Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding HCSRPs. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each HCSRPs were identified, and column 4 shows the cDNA
25 libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. The Incyte clones in column 5 were used to assemble the consensus nucleotide sequence of each HCSRPs and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention:
30 column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows homologous sequences as identified by BLAST analysis; and column 7 shows analytical methods used to characterize each polypeptide through sequence homology and protein motifs. In

particular, the amino acid sequence of SEQ ID NO:1 from about amino acid residue 30 to about 81 is distinct from the tethered ligand thrombin receptor agonist peptide of the N-terminus of the human thrombin receptor and the amino acid sequence of SEQ ID NO:2 from about amino acid residue 115 to about 140 is distinct from the C-terminus joining and constant regions of the human TCR α subunit.

5 The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding HCSR. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:14-26 and to distinguish between SEQ ID NO:14-26 and related polynucleotide sequences. The
10 polypeptides encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express HCSR as a fraction of total tissues expressing HCSR. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing HCSR as a fraction of total tissues expressing HCSR. Column 5 lists the vectors used to subclone each cDNA library. Of particular note is the expression of HCSR in cancer, autoimmune and inflammatory
15 response, and in lung, thymus, bladder, seminal vesicle, and penile tissues, and in rheumatoid arthritis. In addition, SEQ ID NO: 14 is expressed primarily in tumor-associated epithelial tissues and SEQ ID NO: 15 is expressed primarily in growth- and tumor-associated epithelial tissues and in immune response tissues.

 The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries
20 from which cDNA clones encoding HCSR were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

 The invention also encompasses HCSR variants. A preferred HCSR variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid
25 sequence identity to the HCSR amino acid sequence, and which contains at least one functional or structural characteristic of HCSR.

 The invention also encompasses polynucleotides which encode HCSR. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:14-26, which encodes HCSR.

30 The invention also encompasses a variant of a polynucleotide sequence encoding HCSR. In particular, such a variant polynucleotide sequence will have at least about 80%, or alternatively at least about 90%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding HCSR. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID

NO:14-26 which has at least about 80%, or alternatively at least about 90%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:14-26. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of HCSR P.

5 It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HCSR P, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These
10 combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HCSR P, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HCSR P and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring HCSR P under appropriately
15 selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HCSR P or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the
20 nucleotide sequence encoding HCSR P and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode HCSR P and HCSR P derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the
25 synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HCSR P or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID
30 NO:14-26 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of

the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (Perkin-Elmer). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (Perkin-Elmer), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding HCSRП may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence
5 into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the
10 emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

15 In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HCSRП may be cloned in recombinant DNA molecules that direct expression of HCSRП, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express HCSRП.

20 The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HCSRП-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-
25 mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding HCSRП may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223; and Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232.)
30 Alternatively, HCSRП itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of HCSRП, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other

proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) *Methods Enzymol.* 182:392-421.)

The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing.
5 (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY.)

In order to express a biologically active HCSR, the nucleotide sequences encoding HCSR or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding
10 sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding HCSR. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HCSR. Such signals include the ATG initiation codon and adjacent sequences,
15 e.g. the Kozak sequence. In cases where sequences encoding HCSR and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and
20 initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HCSR and appropriate transcriptional and translational
25 control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

30 A variety of expression vector/host systems may be utilized to contain and express sequences encoding HCSR. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or

tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding HCSRPs. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HCSRPs can be achieved using a multifunctional *E. coli* vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSORT1 plasmid (Life Technologies). Ligation of sequences encoding HCSRPs into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for *in vitro* transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of HCSRPs are needed, e.g. for the production of antibodies, vectors which direct high level expression of HCSRPs may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

15 Yeast expression systems may be used for production of HCSRPs. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 20 1995, supra; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; and Scorer, C.A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of HCSRPs. Transcription of sequences encoding HCSRPs may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HCSR_P may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain

infective virus which expresses HCSR_P in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

5 Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

10 For long term production of recombinant proteins in mammalian systems, stable expression of HCSR_P in cell lines is preferred. For example, sequences encoding HCSR_P can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media
15 before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These
20 include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* and *ap^r* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat*
25 confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins
30 (GFP; Clontech), β glucuronidase and its substrate β -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is

also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding HCSR_P is inserted within a marker gene sequence, transformed cells containing sequences encoding HCSR_P can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HCSR_P under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding HCSR and that express HCSR may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of HCSRPs using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HCSRPs is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press. St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HCSRPs include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HCSRPs, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HCSRPs may be cultured under

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HCSRPs may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HCSRPs protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of HCSRPs activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HCSRPs encoding sequence and the heterologous protein sequence, so that HCSRPs may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled HCSR_P may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These

systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, ^{35}S -methionine.

Fragments of HCSRП may be produced not only by recombinant means, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, *supra*, pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A peptide synthesizer (Perkin-Elmer). Various fragments of HCSRП may be synthesized separately and then combined to produce the full length molecule.

THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of HCSRП and human cell surface receptor proteins. In addition, the expression of HCSRП is closely associated with lung, thymus, bladder, seminal vesicle, and penile tissues, with rheumatoid arthritis, and with inflammation, cancer, and the nervous system. Therefore, HCSRП appears to play a role in cell proliferative disorders, immune system disorders, infections, and neuronal disorders. In the treatment of disorders associated with increased HCSRП expression or activity, it is desirable to decrease the expression or activity of HCSRП. In the treatment of disorders associated with decreased HCSRП expression or activity, it is desirable to increase the expression or activity of HCSRП.

Therefore, in one embodiment, HCSRП or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HCSRП. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an immune system disorder such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome,

- episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus,
- 5 systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, trauma, and hematopoietic cancer including lymphoma, leukemia, and myeloma; an infection caused by a viral agent classified as adenovirus, arenavirus, bunyavirus, calicivirus, coronavirus, filovirus, hepadnavirus, herpesvirus, flavivirus, orthomyxovirus, parvovirus, papovavirus, paramyxovirus,
- 10 picornavirus, poxvirus, reovirus, retrovirus, rhabdovirus, or togavirus; an infection caused by a bacterial agent classified as pneumococcus, staphylococcus, streptococcus, bacillus, corynebacterium, clostridium, meningococcus, gonococcus, listeria, moraxella, kingella, haemophilus, legionella, bordetella, gram-negative enterobacterium including shigella, salmonella, or campylobacter, pseudomonas, vibrio, brucella, francisella, yersinia, bartonella, norcardium, actinomyces,
- 15 mycobacterium, spirochaetale, rickettsia, chlamydia, or mycoplasma; an infection caused by a fungal agent classified as aspergillus, blastomyces, dermatophytes, cryptococcus, coccidioides, malassezia, histoplasma, or other fungal agents causing various mycoses; an infection caused by a parasite classified as plasmodium or malaria-causing, parasitic entamoeba, leishmania, trypanosoma, toxoplasma, pneumocystis carinii, intestinal protozoa such as giardia, trichomonas, tissue nematodes
- 20 such as trichinella, intestinal nematodes such as ascaris, lymphatic filarial nematodes, trematodes such as schistosoma, or cestodes such as tapeworm; and a neuronal disorder such as akathisia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, cerebral neoplasms, dementia, depression, diabetic neuropathy, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, peripheral neuropathy, multiple sclerosis,
- 25 neurofibromatosis, Parkinson's disease, paranoid psychoses, postherpetic neuralgia, schizophrenia, and Tourette's disorder.

In another embodiment, a vector capable of expressing HCSR or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HCSR including, but not limited to, those described above.

- 30 In a further embodiment, a pharmaceutical composition comprising a substantially purified HCSR in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HCSR including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of HCSR may be

administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HCSRП including, but not limited to, those listed above.

In a further embodiment, an antagonist of HCSRП may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HCSRП. Examples of such disorders include, but are not limited to, those cell proliferative disorders, immune system disorders, 5 infections, and neuronal disorders described above. In one aspect, an antibody which specifically binds HCSRП may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express HCSRП.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding HCSRП may be administered to a subject to treat or prevent a disorder associated with 10 increased expression or activity of HCSRП including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made 15 by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of HCSRП may be produced using methods which are generally known in the art. In particular, purified HCSRП may be used to produce antibodies or to screen libraries of 20 pharmaceutical agents to identify those which specifically bind HCSRП. Antibodies to HCSRП may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit 25 dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with HCSRП or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral 30 gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Cornebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to HCSRП have an amino acid sequence consisting of at least about 5 amino acids, and generally will

consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HCSRPs amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to HCSRPs may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) *Nature* 256:495-497; Kozbor, D. et al. (1985) *J. Immunol. Methods* 81:31-42; Cote, R.J. et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:2026-2030; and Cole, S.P. et al. (1984) *Mol. Cell Biol.* 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Neuberger, M.S. et al. (1984) *Nature* 312:604-608; and Takeda, S. et al. (1985) *Nature* 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HCSRPs-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) *Proc. Natl. Acad. Sci. USA* 88:10134-10137.)

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:3833-3837; Winter, G. et al. (1991) *Nature* 349:293-299.)

Antibody fragments which contain specific binding sites for HCSRPs may also be generated. For example, such fragments include, but are not limited to, $F(ab')_2$ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) *Science* 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HCSRPs and its

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of HCSR-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, *supra*, and Coligan et al. *supra*.)

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Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding HCSRPs. (See, e.g., Sambrook, supra; Ausubel, 1995, supra.)

Genes encoding HCSRPs can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding HCSRPs. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding HCSRPs. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may be employed. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HCSRPs.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary

number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable
5 pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA).

Pharmaceutical compositions for oral administration can be formulated using
10 pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active
compounds with solid excipient and processing the resultant mixture of granules (optionally, after
15 grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents
20 may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures.
25 Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or
30 starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's

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Normal or standard values for HCSRП expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to HCSRП under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of HCSRП expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HCSRП may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of HCSRП may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of HCSRП, and to monitor regulation of HCSRП levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HCSRП or closely related molecules may be used to identify nucleic acid sequences which encode HCSRП. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding HCSRП, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the HCSRП encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:14-26 or from genomic sequences including promoters, enhancers, and introns of the HCSRП gene.

Means for producing specific hybridization probes for DNAs encoding HCSRП include the cloning of polynucleotide sequences encoding HCSRП or HCSRП derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding HCSRП may be used for the diagnosis of disorders associated with expression of HCSRП. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal

hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an immune system disorder such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, trauma, and hematopoietic cancer including lymphoma, leukemia, and myeloma; an infection caused by a viral agent classified as adenovirus, arenavirus, bunyavirus, calicivirus, coronavirus, filovirus, hepadnavirus, herpesvirus, flavivirus, orthomyxovirus, parvovirus, papovavirus, paramyxovirus, picornavirus, poxvirus, reovirus, retrovirus, rhabdovirus, or togavirus; an infection caused by a bacterial agent classified as pneumococcus, staphylococcus, streptococcus, bacillus, corynebacterium, clostridium, meningococcus, gonococcus, listeria, moraxella, kingella, haemophilus, legionella, bordetella, gram-negative enterobacterium including shigella, salmonella, or campylobacter, pseudomonas, vibrio, brucella, francisella, yersinia, bartonella, norcardium, actinomyces, mycobacterium, spirochaetale, rickettsia, chlamydia, or mycoplasma; an infection caused by a fungal agent classified as aspergillus, blastomyces, dermatophytes, cryptococcus, coccidioides, malassezia, histoplasma, or other fungal agents causing various mycoses; an infection caused by a parasite classified as plasmodium or malaria-causing, parasitic entamoeba, leishmania, trypanosoma, toxoplasma, pneumocystis carinii, intestinal protozoa such as giardia, trichomonas, tissue nematodes such as trichinella, intestinal nematodes such as ascaris, lymphatic filarial nematodes, trematodes such as schistosoma, or cestodes such as tapeworm; and a neuronal disorder such as akathisia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder,

catatonia, cerebral neoplasms, dementia, depression, diabetic neuropathy, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, peripheral neuropathy, multiple sclerosis, neurofibromatosis, Parkinson's disease, paranoid psychoses, postherpetic neuralgia, schizophrenia, and Tourette's disorder. The polynucleotide sequences encoding HCSRП may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered HCSRП expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HCSRП may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding HCSRП may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding HCSRП in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of HCSRП, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding HCSRП, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance

of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HCSR
5 HCSR may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding HCSR, or a fragment of a polynucleotide complementary to the polynucleotide encoding HCSR, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or
10 quantification of closely related DNA or RNA sequences.

Methods which may also be used to quantify the expression of HCSR include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be
15 accelerated by running the assay in a high-throughput format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray
20 can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g.,
25 Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding HCSR may be
30 used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price,

C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the
5 Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding HCSRP on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

10 In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides
15 valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be
20 used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, HCSRP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a
25 solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between HCSRP and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are
30 synthesized on a solid substrate. The test compounds are reacted with HCSRP, or fragments thereof, and washed. Bound HCSRP is then detected by methods well known in the art. Purified HCSRP can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HCSRП specifically compete with a test compound for binding HCSRП. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HCSRП.

5 In additional embodiments, the nucleotide sequences which encode HCSRП may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding
10 description, utilize the present invention to its fullest extent. The following embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific
15 embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. [Attorney Docket No. PF-0636 P, filed November 12, 1998], U.S. Ser. No. [Attorney Docket No. PF-0650 P, filed December 7, 1998], and U.S. Ser. No. 60/123,404 are hereby
20 expressly incorporated by reference.

EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some
25 tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

30 Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA

purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERSCRIPIT plasmid system (Life Technologies), using the
5 recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column
10 chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSFORT1 plasmid (Life Technologies), or pINCY (Incyte Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 α , DH10B, or
15 ElectroMAX DH10B from Life Technologies.

II. Isolation of cDNA Clones

Plasmids were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge
20 Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a
25 high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

30 III. Sequencing and Analysis

cDNA sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Perkin-Elmer) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared

using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the
5 ABI PRISM 373 or 377 sequencing system (Perkin-Elmer) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example V.

10 The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third
15 column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software
20 (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA
25 sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled
30 into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS,

DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:14-26. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; Ausubel, 1995, *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding HCSRП occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

V. Extension of HCSR Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:14-26 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other
 5 primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

10 Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+} , $(NH_4)_2SO_4$,
 15 and β -mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2:
 20 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 μ l PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 μ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar,
 25 Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 μ l to 10 μ l aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

30 The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones

were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequences of SEQ ID NO:14-26 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:14-26 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μ Ci of [γ -³²P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10⁷ counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and

compared.

VII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) *Science* 270:467-470; Shalon, D. et al. (1996) *Genome Res.* 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

VIII. Complementary Polynucleotides

Sequences complementary to the HCSR-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring HCSR. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of HCSR. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the HCSR-encoding transcript.

IX. Expression of HCSR_P

Expression and purification of HCSRP is achieved using bacterial or virus-based expression systems. For expression of HCSRP in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA

transcription. Examples of such promoters include, but are not limited to, the *trp-lac (tac)* hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3).

Antibiotic resistant bacteria express HCSRП upon induction with isopropyl beta-D-

5 thiogalactopyranoside (IPTG). Expression of HCSRП in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding HCSRП by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong
10 polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

15 In most expression systems, HCSRП is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham
20 Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from HCSRП at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995,
25 supra, ch. 10 and 16). Purified HCSRП obtained by these methods can be used directly in the following activity assay.

X. Demonstration of HCSRП Activity

An assay for HCSRП activity measures the expression of HCSRП on the cell surface. cDNA encoding HCSRП is transfected into an appropriate mammalian cell line. Cell surface proteins are
30 labeled with biotin as described (de la Fuente, M. A. et al. (1997) Blood 90:2398-2405). Immunoprecipitations are performed using HCSRП-specific antibodies, and immunoprecipitated samples are analyzed using SDS-PAGE and immunoblotting techniques. The ratio of labeled immunoprecipitant to unlabeled immunoprecipitant is proportional to the amount of HCSRП expressed on the cell surface.

An alternative assay for HCSR activity is based on a prototypical assay for ligand/receptor-mediated modulation of cell proliferation. This assay measures the amount of newly synthesized DNA in Swiss mouse 3T3 cells expressing HCSR. An appropriate mammalian expression vector containing cDNA encoding HCSR is added to quiescent 3T3 cultured cells using transfection methods well known in the art. The transfected cells are incubated in the presence of [³H]thymidine and varying amounts of HCSR ligand. Incorporation of [³H]thymidine into acid-precipitable DNA is measured over an appropriate time interval using a tritium radioisotope counter, and the amount incorporated is directly proportional to the amount of newly synthesized DNA. A linear dose-response curve over at least a hundred-fold HCSR ligand concentration range is indicative of receptor activity. One unit of activity per milliliter is defined as the concentration of HCSR producing a 50% response level, where 100% represents maximal incorporation of [³H]thymidine into acid-precipitable DNA. (McKay, I. and Leigh, I., eds. (1993) Growth Factors: A Practical Approach, Oxford University Press, New York, NY, p. 73.)

XI. Functional Assays

HCSR function is assessed by expressing the sequences encoding HCSR at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10 μ g of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2 μ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in

flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of HCSRП on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding HCSRП and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding HCSRП and other genes of interest can be analyzed by northern analysis or microarray techniques.

10 XII. Production of HCSRП Specific Antibodies

HCSRП substantially purified using polyacrylamide gel electrophoresis (PAGE: see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the HCSRП amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-HCSRП activity by, for example, binding the peptide or HCSRП to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

25 XIII. Purification of Naturally Occurring HCSRП Using Specific Antibodies

Naturally occurring or recombinant HCSRП is substantially purified by immunoaffinity chromatography using antibodies specific for HCSRП. An immunoaffinity column is constructed by covalently coupling anti-HCSRП antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HCSRП are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HCSRП (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt

antibody/HCSRП binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and HCSRП is collected.

XIV. Identification of Molecules Which Interact with HCSRП

HCSRП, or biologically active fragments thereof, are labeled with ¹²⁵I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) Biochem. J. 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HCSRП, washed, and any wells with labeled HCSRП complex are assayed. Data obtained using different concentrations of HCSRП are used to calculate values for the number, affinity, and association of HCSRП with the candidate molecules.

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

| Polypeptide SEQ ID NO: | Nucleotide SEQ ID NO: | Clone ID | Library | Fragments |
|---------------------------|--------------------------|----------|-----------|--|
| 1 | 14 | 2355971 | LUNGNOT20 | 2355971F6 and 2355971H1 (LUNGNOT20) |
| 2 | 15 | 2917059 | THYMFET03 | 2917059F6, 2917059H1, and 2917059T6 (THYMFET03) |
| 3 | 16 | 538181 | LNODNOT02 | 538181H1 (LNODNOT02), 1437379F1 (PANCNOT08), 2945139F6 (BRAITUT23), SAJA00666R1, SAJA00920R1, SAJA00173F1, SAJA00783R1 |
| 4 | 17 | 1368760 | SCORNON02 | 1368760H1 and 1368760T6 (SCORNON02), SAUB01170F1 |
| 5 | 18 | 1670669 | BMARNOT03 | 1670669F6 and 1670669H1 (BMARNOT03), SAEA03216R1 |
| 6 | 19 | 2851578 | BRSTTUT13 | 992052T1 (COLNNOT11), 1579409F1 (DUODNOT01), 1880543T6 (LEUKNOT03), 4088508H1 (LIVRNOT06), 2851578H1, 2851578X310F1, and 2851578X311F1 (BRSTTUT13) |
| 7 | 20 | 3393757 | LUNGNOT28 | 318405H1 (EOSIHET02), 2230506H1 (PROSNOT16), 4592475F6 (MASTTX01), 3393757H1, 3393757X301D2, and 3393757X305B2 (LUNGNOT28), SZAH04055F1, SZAH01718F1 |
| 8 | 21 | 312256 | LUNGNOT02 | 306245T6 (HEARNOT01), 312256H1 (LUNGNOT02), 4175078H1 (SINTNOT21), SCAA06277V1, SCAA02060V1, SCAA02643V1 |
| 9 | 22 | 1615704 | BRAITUT12 | 1615704F6 (BRAITUT12), 1615704H1 (BRAITUT12), 1615704X13 (BRAITUT12), 1727857F6 (PROSNOT14), 3539783H1 (SEMVNOT04) |
| 10 | 23 | 1659465 | URETTUT01 | 265886F1 (HNT2AGT01), 1441970R1 (THYRNOT03), 1522238X12 (BLADTUT04), 1659465H1 (URETTUT01), 2187904T6 (PROSNOT26), 3237635H1 (COLAUCT01) |

Table 1 (cont.)

| Polypeptide SEQ ID NO: | Nucleotide SEQ ID NO: | Clone ID | Library | Fragments |
|---------------------------|--------------------------|----------|-----------|---|
| 11 | 24 | 2120743 | BRSTNOT07 | 661461R6 (BRAINTOT03), 1318546F6 (BLADNOT04), 1318694T1 (BLADNOT04), 2044109H1 (HIPONON02), 2120743F6 (BRSTNOT07), 2120743H1 (BRSTNOT07), SBIA06480D1, SBIA02214D1, SBIA05533D1, SBIA03320D1, SBIA10018D1, SBIA07320D1, SBIA00175D1 |
| 12 | 25 | 3344986 | SPLNNOT09 | 138687F1 (LIVRNOT01), 138687X27C1 (LIVRNOT01), 169384X8 (EMARNOR02), 3344986H1 (SPLNNOT09) |
| 13 | 26 | 3576503 | BRONNOT01 | 2525456F6 (BRAITUT21), 2527260F6 (BRAITUT21), 3576503H1 (BRONNOT01), 3742127F6 (MENTNOT01) |

Table 2

| Polypeptide SEQ ID NO: | Amino Acid Residues | Potential Phosphorylation Sites | Potential Glycosylation Sites | Motifs, Signature Sequences, and Protein Domains | Homologous Sequences | Analytical Methods |
|---------------------------|------------------------|--|-------------------------------------|---|--|--|
| 1 | 81 | S21 T43 | | Signal peptide: M1-A22 Receptor signature: C12-R28 | thrombin receptor g339677 | BLAST MOTIFS BLOCKS PRINTS HMM |
| 2 | 140 | S20 S33 S49 S73 S61 T90 | N88 | Signal peptide: M1-G22 Receptor tyrosine kinase signature: R82-A99 Receptor signature: A99-C111 | T cell receptor alpha chain g1223888 | BLAST MOTIFS BLOCKS PRINTS HMM |
| 3 | 358 | T47 T65 T163 T228 T283 S299 T352 | | Ig domains: G91-G144; G184-A241 Signal peptide: M1-A25 | IgG Fc receptor g583604 | BLAST MOTIFS PFAM SPSCAN HMM |

Table 2 (cont.)

| Polypeptide SEQ ID NO: | Amino Acid Residues | Potential Phosphorylation Sites | Potential Glycosylation Sites | Motifs, Signature Sequences, and Protein Domains | Homologous Sequences | Analytical Methods |
|---------------------------|------------------------|--|-------------------------------------|---|---|--|
| 4 | 201 | T44 T131 T65 T167 | N63 | Glutamate receptor domain: M1-G170 GPCR/Neurotransmitter receptor signatures: T114-T122; M7-S26 L145-H155; F90-G103 Signal peptide: M1-G28 | Glutamate receptor isoform g507827 | BLAST MOTIFS PFAM PRINTS SPSCAN |
| 5 | 117 | T54 T75 S104 | | Cytokine receptor signature: D32-E93 Signal peptide: M1-W24 or M1-A25 | EPO receptor isoform g553281 | BLAST MOTIFS PROFILESAN SPSCAN HMM |
| 6 | 455 | S22 S380 S53 T189 T206 S271 S281 T310 S430 | N76 N207 | Rhodopsin GPCR domain: G141-Y387 Rhodopsin signatures: F126-C150; N159-R180 L204-V226; A325-A349 F369-L395 Transmembrane domains: L291-T310; A128-C150 | Rhodopsin-like GPCR/HM74 g219867 | BLAST MOTIFS PROFILESAN PFAM BLOCKS PRINTS HMM |

Table 2 (cont.)

| Polypeptide SEQ ID NO: | Amino Acid Residues | Potential Phosphorylation Sites | Potential Glycosylation Sites | Motifs, Signature Sequences, and Protein Domains | Homologous Sequences | Analytical Methods |
|---------------------------|------------------------|--|---|---|---|---|
| 7 | 453 | S71 T418 S87 T244 T340 S428 T433 T438 T449 | N73 N77 N183 N247 N252 | Secretin signatures: V155-K179; I218-L241 K261-L286; W303-K328 A377-L398 Transmembrane domains: M159-L177; I302-V322 Y378-L398 | Secretin-like GPCR/EMR1 g784994 | BLAST MOTIFS BLOCKS PRINTS HMM |
| 8 | 442 | T53 S103 S115 T155 S190 T176 T233 T310 S368 Y241 Y329 | N67 N101 N113 N165 N304 N308 N432 | Transmembrane domain: A375-I394 Signal peptide: M1-G44 Immunoglobulin domains: G57-L126; G159-V222 G260-A315 | Immuno- superfamily protein B12 g3779242 | HMM BLAST PFAM SPSCAN BLOCKS-DOMO |
| 9 | 382 | S152 S199 T267 T324 S29 | N65 N178 N322 | Transmembrane domains: M1-I21; L289-F306 Signal peptide M1-S29 Somatostatin receptor: L237-L254 2 Poly-Immunoglobulin receptor: F9-P46; E86-P123 P104-F134; V259-F306 L283-A320 | | HMM SPSCAN PRINTS BLOCKS-DOMO |

Table 2 (cont.)

| Polypeptide SEQ ID NO: | Amino Acid Residues | Potential Phosphorylation Sites | Potential Glycosylation Sites | Motifs, Signature Sequences, and Protein Domains | Homologous Sequences | Analytical Methods |
|---------------------------|------------------------|--|-------------------------------------|---|-------------------------|--|
| 10 | 257 | S162 S7 T228 | | Transmembrane domain: Y187-L210 Signal peptide: M1-S33 Endothelin-A receptor: Q236-T255 2 Poly-Immunoglobulin receptor: Y91-R138; Y170-L217 | | HMM SPSCAN PRINTS BLOCKS-DOMO |
| 11 | 697 | S266 S623 T18 S138 T155 S215 S247 T524 T631 T684 S4 S32 S113 S122 T219 T249 T332 T519 T606 T611 S619 | N111 N533 N598 | Transmembrane domains: L36-V54; S62-Y84 Y125-R142; S177-I192 S198-F214; F298-H320 I381-G398 Signal peptide: M1-W56 Prostanoid EP1 receptor: V360-S384; D110-R134 2 Poly-Immunoglobulin receptor: I64-N111; L162-A209 | | HMM SPSCAN PRINTS BLOCKS-DOMO |

| Polypeptide SEQ ID NO: | Amino Acid Residues | Potential Phosphorylation Sites | Potential Glycosylation Sites | Motifs, Signature Sequences, and Protein Domains | Homologous Sequences | Analytical Methods |
|---------------------------|------------------------|--|-------------------------------------|--|---|---|
| 12 | 325 | S56 T185 T234 S289 S76 S211 T245 S248 | N64 N287 | Transmembrane domain: Q26-V44 Signal peptide: M1-S52 2 Poly-Immunoglobulin receptor: M1-P48; A22-K69 V95-G142; V118-G165 | Non-CD4 glycoprotein gp120 receptor R32188 | HMM BLAST SPSCAN BLOCKS-DOMO |
| 13 | 369 | T12 T69 T299 S312 S330 T346 S50 S92 T145 | N10 N90 N297 | Transmembrane domains: I144-L161; V196-L219 L264-Y283 Signal peptide: M1-G47 Prostaglandin D receptor: Y6-A20; L41-P58 L93-L104; I185-S200 P236-E259; P280-K291 2 Poly-Immunoglobulin receptor: T11-P58; N10-L57 R52-A99; S195-L225 7 Transmembrane receptor: G33-P280 | DP prostanoid receptor g940379 | HMM BLAST SPSCAN PRINTS BLOCKS-DOMO PFAM |

Table 3

| Nucleotide SEQ ID NO: | Selected Fragments | Tissue Expression (Fraction of Total) | Diseases, Disorders, or Conditions (Fraction of Total) | Vector |
|--------------------------|-----------------------|--|--|-------------|
| 14 | 146-301 | Lung (1.000) | Cancer (1.000) | pINCY |
| 15 | 423-500 | Fetal Thymus (0.333) Wrist Synovium (0.333) Seminal Vesicle (0.333) | Cancer (0.333) Cell Proliferation (0.333) Inflammation (0.333) | pINCY |
| 16 | 94-138 | Hematopoietic/Immune (0.636) Gastrointestinal (0.273) | Inflammation (0.636) Cancer (0.364) | PSPORT1 |
| 17 | 703-747 | Nervous System (1.000) | Neurological (0.333) Trauma (0.333) Cancer (0.333) | PSPORT1 |
| 18 | 293-325 434-478 | Cardiovascular (0.333) Developmental (0.167) Hematopoietic/Immune (0.167) | Cancer (0.833) Fetal (0.167) | pINCY |
| 19 | 109-153 1339-1383 | Gastrointestinal (0.333) Reproductive (0.333) Hematopoietic/Immune (0.250) | Cancer (0.500) Inflammation (0.250) Fetal (0.167) | pINCY |
| 20 | 519-563 | Hematopoietic/Immune (0.571) Cardiovascular (0.143) Reproductive (0.143) | Inflammation (0.357) Cancer (0.214) Trauma (0.143) | pINCY |
| 21 | 319-360 | Nervous (0.235) Cardiovascular (0.206) Reproductive (0.206) | Cancer and Cell Proliferation (0.529) Inflammation (0.412) | PBLUESCRIPT |
| 22 | 558-599 | Reproductive (0.361) Nervous (0.180) Cardiovascular (0.115) | Cancer and Cell Proliferation (0.689) Inflammation (0.311) | pINCY |

Table 3 (cont.)

| Nucleotide SEQ ID NO: | Selected Fragments | Tissue Expression (Fraction of Total) | Diseases, Disorders, or Conditions (Fraction of Total) | Vector |
|--------------------------|-----------------------|--|---|--------|
| 23 | 8-47 900-940 | Reproductive (0.280) Nervous (0.160) Gastrointestinal (0.133) | Cancer and Cell Proliferation (0.666) Inflammation (0.253) | pINCY |
| 24 | 800-840 | Reproductive (0.237) Nervous (0.186) Cardiovascular (0.153) | Cancer and Cell Proliferation (0.664) Trauma (0.186) | pINCY |
| 25 | 770-814 | Hematopoietic/Immune (0.333) Gastrointestinal (0.222) Cardiovascular (0.111) Endocrine (0.111) Nervous (0.111) Reproductive (0.111) | Inflammation (0.444) Fetal (0.222) Trauma (0.222) | pINCY |
| 26 | 1078-1119 | Nervous (0.500) Cardiovascular (0.250) Musculoskeletal (0.250) | Cancer (0.750) Trauma (0.250) | pINCY |

Table 4

| Polypeptide SEQ ID NO: | Library | Library Description |
|---------------------------|-----------|---|
| 14 | LUNGNOT20 | This library was constructed using RNA isolated from right upper lobe lung tissue removed from a 61-year-old Caucasian male. Pathology indicated panacinal emphysema with blebs in the right anterior upper lobe and apex, as well as emphysema in the right posterior upper lobe. Patient history included angina pectoris and gastric ulcer. Family history included a subdural hemorrhage, cancer, atherosclerotic coronary artery disease, and pneumonia. |
| 15 | THYMFET03 | This library was constructed using RNA isolated from thymus tissue removed from a premature Caucasian male fetus who died at birth. |
| 16 | LNODNOT02 | This library was constructed using RNA isolated from the lymph node tissue of a 42-year-old Caucasian female who died of cardiac arrest. |
| 17 | SCORNON02 | This normalized spinal cord library was constructed from 3.24 million independent clones from a spinal cord library. RNA was isolated from spinal cord tissue removed from a 71-year-old Caucasian male who died from respiratory arrest. Patient history included myocardial infarction, gangrene, and end stage renal disease. The normalization and hybridization conditions were adapted from Soares et al. (PNAS (1994) 91:9928). |
| 18 | BMARNOT03 | This library was constructed using RNA isolated from left tibial bone marrow tissue of a 16-year-old Caucasian male during a partial left tibial osteotomy with free skin graft. Patient history included an abnormality of the red blood cells. Previous surgeries included bone and bone marrow biopsy and soft tissue excision. Family history included osteoarthritis. |
| 19 | BRSTTUT13 | This library was constructed using RNA isolated from breast tumor tissue removed from a 46-year-old Caucasian female during a unilateral extended simple mastectomy with breast reconstruction. Pathology indicated an invasive grade 3 adenocarcinoma, ductal type with apocrine features and greater than 50% intraductal component. Patient history included breast cancer. |

Table 4 (cont.)

| Polypeptide SEQ ID NO: | Library | Library Description |
|---------------------------|-----------|--|
| 20 | LUNGNOT28 | This library was constructed using RNA isolated from lung tissue removed from a 53-year-old male. Pathology for the associated tumor tissue indicated grade 4 adenocarcinoma. |
| 21 | LUNGNOT02 | This library was constructed using RNA isolated from the lung tissue of a 47-year-old Caucasian male, who died of a subarachnoid hemorrhage. |
| 22 | BRAITUT12 | This library was constructed using RNA isolated from brain tumor tissue removed from the left frontal lobe of a 40-year-old Caucasian female during excision of a cerebral meningeal lesion. Pathology indicated grade 4 gemistocytic astrocytoma. |
| 23 | URETTUT01 | This library was constructed using RNA isolated from right ureter tumor tissue of a 69-year-old Caucasian male during ureterectomy and lymph node excision. Pathology indicated invasive grade 3 transitional cell carcinoma. Patient history included benign colon neoplasm, asthma, emphysema, acute duodenal ulcer, and hyperplasia of the prostate. Family history included atherosclerotic coronary artery disease, congestive heart failure, and malignant lung neoplasm. |
| 24 | BRSTNOT07 | This library was constructed using RNA isolated from diseased breast tissue removed from a 43-year-old Caucasian female during a unilateral extended simple mastectomy. Pathology indicated mildly proliferative fibrocystic changes with epithelial hyperplasia, papillomatosis, and duct ectasia. Pathology for the associated tumor tissue indicated invasive grade 4, nuclear grade 3 mammary adenocarcinoma with extensive comedo necrosis. Family history included epilepsy, cardiovascular disease, and type II diabetes. |
| 25 | SPLNNOT09 | This library was constructed using RNA isolated from diseased spleen tissue removed from a 22-year-old Caucasian male during a total splenectomy. Pathology indicated Gaucher's disease with marked splenomegaly. Patient history included thyroid disorders and type 1 Gaucher's disease. |

Table 4 (cont.)

| Polypeptide SEQ ID NO: | Library | Library Description |
|---------------------------|----------|--|
| 26 | BRNNOT01 | This library was constructed using RNA isolated from bronchial tissue removed from a 15-year-old Caucasian male. |

Table 5

| Program | Description | Reference | Parameter Threshold |
|-------------------|---|--|--|
| ABI FACTURA | A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences. | Perkin-Elmer Applied Biosystems, Foster City, CA. | |
| ABI/PARACEL FDF | A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences. | Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA. | Mismatch <50% |
| ABI AutoAssembler | A program that assembles nucleic acid sequences. | Perkin-Elmer Applied Biosystems, Foster City, CA. | |
| BLAST | A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx. | Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402. | ES7's: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less |
| FASTA | A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch. | Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489. | ES7's: fasta E value=1.0E-6 Assembled ES7's: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less Full Length sequences: fastx score=100 or greater |
| BLIMPS | A BLOCKS IMPROVED Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions. | Henikoff, S. and J.G. Henikoff, Nucl. Acid Res., 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424. | Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and, if applicable, Probability value= 1.0E-3 or less |
| HMMER | An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM. | Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322. | Score=10-50 bits for PFAM hits, depending on individual protein families |

Table 5 (cont.)

| Program | Description | Reference | Parameter Threshold |
|-------------|---|--|--|
| ProfileScan | An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite. | Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221. | Normalized quality score ₂ GCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1. |
| Phred | A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability. | Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194. | |
| Phrap | A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences. | Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA. | Score= 120 or greater; Match length= 56 or greater |
| Consed | A graphical tool for viewing and editing Phrap assemblies | Gordon, D. et al. (1998) Genome Res. 8:195-202. | |
| SPScan | A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides. | Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439. | Score=3.5 or greater |
| Motifs | A program that searches amino acid sequences for patterns that matched those defined in Prosite. | Bairoch et al. <i>supra</i> ; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI. | |